as Vincent Allfrey stated, "should so approximate the soluble phase of the cytoplasm that the cell particulates remain morphologically, structurally, and functionally intact." But, he added, "Such a medium has not yet been devised, and in practice all isolation media introduce more or less serious alterations in both structure and function" (1959, p. 202).

The challenge, then, was to figure out which medium would most closely correspond to the environment in living cells. Initially a saline solution seemed physiologically realistic, and Claude employed it in his early fractionation studies. However, this medium caused clumping and agglutination of the cytoplasmic particulates and failed to preserve the morphological integrity of organelles when compared with micrographs of whole cells. As noted previously, this led Hogeboom and his collaborators to try a hypertonic 0.88 *M* sucrose medium, which succeeded in preserving the rod-like appearance of mitochondria (Hogeboom et al., 1948). However, the crucial function of ATP synthesis (suspected to be localized in mitochondria) was lost. Accordingly, one of the collaborators, Schneider (1948), explored some of the more commonly used sucrose concentrations. He showed that with an isotonic 0.25 *M* sucrose solution, the mitochondrial fraction would carry out oxidative phosphorylation (though the resemblance of its particles to intact mitochondria was somewhat compromised).[10] Soon the medium in most fractionation studies was selected from the class of isotonic sucrose solutions.[11]

The logic of the argument for preferring this fractionation technique is noteworthy. The claim that the mitochondrion was the locus of oxidative phosphorylation was based primarily on fractionation studies, but the justification for doing fractionation in sucrose and especially in isotonic sucrose was that it produced a fraction that possessed the enzymes for oxidative phosphorylation and could carry out the reactions that realized that function. The

[10] A similar dependence of functional activity on sucrose concentration was found in studies of isolated thymus nuclei – nuclei isolated in 0.25 *M* sucrose synthesized protein and RNA but those prepared in 0.4 *M* sucrose did not (Allfrey, 1959, p. 206). Allfrey also reported a number of other factors that affect the appearance and function of isolated nuclei, including pH and ionic strength, and provided another example of a form/function tradeoff: "It is a curious fact that when calf thymocyte nuclei are isolated in 0.25 *M* sucrose-0.003 *M* $CaCl_2$, they are granular in appearance, but active in many synthetic systems; if they are isolated in a hypertonic medium which preserves optical homogeneity they lose their synthetic capacity" (1959, p. 208).

[11] Witter, Watson, and Cottone (1955) compared electron micrographs so as to examine the fine structure of mitochondrial fractions isolated using various sucrose media and concluded that 0.44 *M* sucrose with pH regulated to 6.2 with citrate produced the best resolution. Based on similar studies, Novikoff (1956a) favored 0.25 *M* sucrose with 7.3% polyvinylpyrrollidone at a pH of 7.6 to 7.8. In general, the strategy was to tweak the method until it generated the clearest or most useful results for the current purpose.